Protocol for Relative Fluorescence Unit and GFP Growth rate measurements

1. Restreak the plates two days before measurement

Construct	Plasmid	Strain
E0240	pSB3K3	DH10B
kdpFp (T)	pSB3K3	DH10B
kdpFp (T>G)	pSB3K3	DH10B
kdpFp (T>C)	pSB3K3	DH10B
kdpFp (T>A)	pSB3K3	DH10B
E0240	pSB3K3	TK2240
kdpFp (T>A)	pSB3K3	TK2240

2. Pick a colony from each plate to start overnight culture in 2mL of K115 minimal medium at 37 in 12mL falcon tubes.
3. Prepare the stock of media of specific K^+ concentration:K0, K0.0015625, K0.003125, K0.00625, 0.0125, K0.025, K0.05, K0.1, K0.2, K0.4 in new falcons and then aliquot1 mL into Corning® 96 well storage system storage block, 2 mL, V-bottom.
4. Wash the cell three times with 0.8% NaCl solution (2mL each). In between each washing step, spin the cells with 3000rpm at 4 for 3 minutes. After washing, resuspend the cells in 700L of fresh K0 medium.
5. Take out 200L of washed cells to check OD595 and then dilute all the suspensions to around 1 (OD595).
6. Take out 25L (1/40 of the medium) of washed cells to mix with K minimal medium of different K concentration in the storage block.
7. Incubate the culture in 37 for 1-2 hours until it reaches the mid-exponential phase (OD600: 0.3-0.5)
8. Take out 200L of the culture from the storage block and put into a micro test plate 96 well flat-bottom. Measure green fluorescence level and OD595 using Envision Multilabel Reader every 15 minutes for three times in total. In between measurements, keep incubating the cells in 37 while shaking.

9. Repeat step 1-8 2 more times with other colonies.

Data Processing for Relative Fluorescence Unit (RFU) Measurement

1. Normalize the OD595 reading by subtracting the average of three blank K0 media from the OD595 measurements. This step is to eliminate the influence of the background absorbance for OD measurement.
2. F stands for the green fluorescence level obtained directly from the plate reader reading, OD595 is the normalized one.

The construct pSB3K3-BBa_E0240 was used as blank for each strain thus all the RFU calculating results can be normalized by subtracting the RFU of the negative control construct in the strain accordingly.

Data Processing for GFP Synthesis Rate Measurement

1. Calculate the RFU and then take the slope of the RFU over time as the GFP synthesis rate for each construct in different K^+ minimal media.
2. Normalize the rate calculation by subtracting the negative control construct's GFP synthesis rate.

References:

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